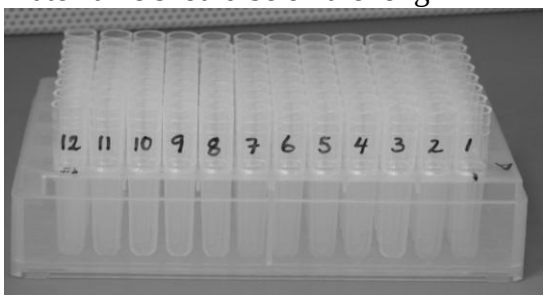
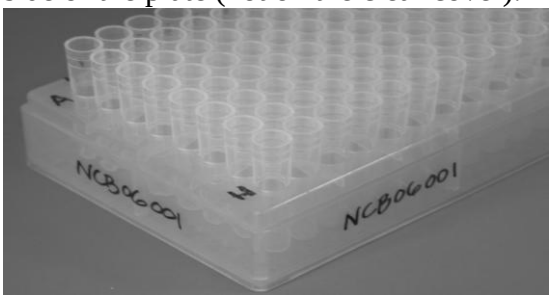
Sample Collection Protocol for Eastern Regional Small Grains Genotyping Lab, Raleigh, NC

One to two week old seedlings at the two leaf stage. The leaves should be fully expanded, not rolled. You can use older or field grown plants, but DNA yields are best from seedling tissue. [We plant in a 128 cell tray from Hummert cat. # 14-3120, but you could also use the 288 cell tray cat. # 14-3124. Be sure to use bottom trays without drainage holes cat. # 14-2865. We use 1 jumbo or triple size cotton ball per cell.]
Transplant the cotton ball directly into soil mix in the greenhouse

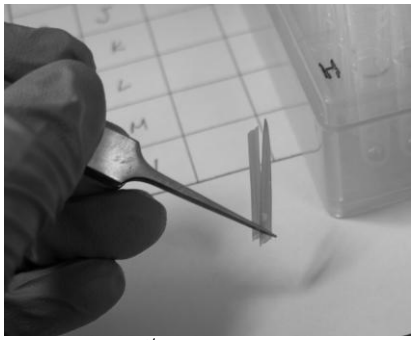
Plate name should be on the long H1-H12 side of the plate (not on the clear cover). A1 should be in the upper left.

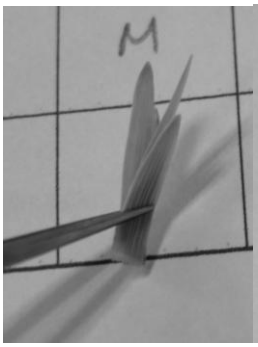


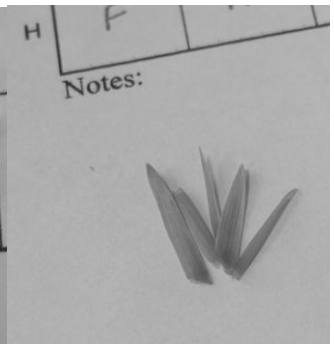


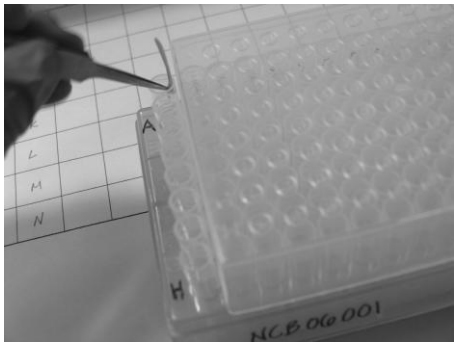
[Plate Name nomenclature = 3 digit breeder code_2 digit year_3 digit plate number]

Collect approximately 25mg of tissue per sample. The first photo is a sample from a single plant.
The next photos are a bulk of five seedlings from a single cell and should be approximately 25mg of tissue, a little bit from each seedling.









After the 1st strip of tubes is collected they are capped and can be taken to the -20°C.
(If you are collecting in the greenhouse or field then ice is a must) After collection plates can be kept at -80°C.

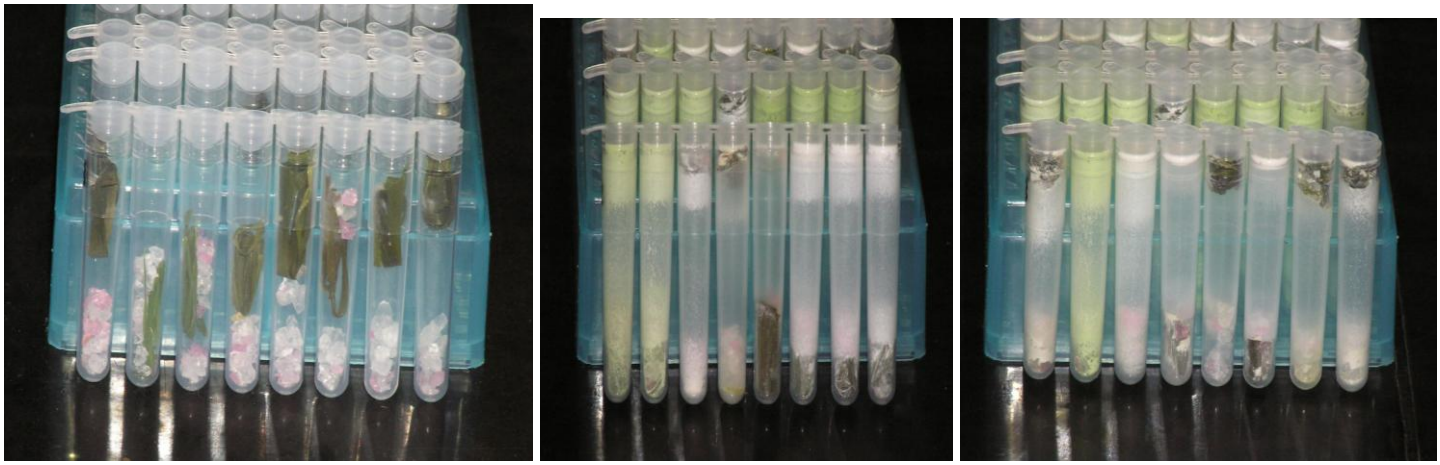
If you are collecting on silica gel, plates are kept at room temperature but out of direct heat or sun.

Remember to give the forceps a quick wipe between each sample with a Kimwipe

Evenly collected tissue is a good thing + Consistency in samples give more uniform DNA yields

The silica gel is a mixture of Blue Silica Gel and Clear Silica Gel. The blue gel will turn pink as it absorbs moisture. Keep the plates sealed up when not in use. If it turns pink, dry it in an oven (up to 65°C) until it is blue again. You always want it to be blue until you collect the tissues. After the tissue is added, it should turn pink as it absorbs the moisture from the leaves and the tissue will become 'dry' looking just as if you were lyophilizing them. You also don't want to have excessive amounts of tissue because you will exceed the silica absorption capacity and your samples will mold!!!

Examples of what happens when there is too much tissue collected:



When there is too much tissue, it starts to mold (it is hard to see here, but E & H are molding) and the samples stay 'juicy' which means they will not grind well and will be degraded. The same thing happens with beads, if there is too much tissue the bead can't get past it to grind it up whether it is lyophilized or frozen. This can mean missing samples and thus data points.